

Biomarker Associations with Efficacy of Abiraterone Acetate and Exemestane in Postmenopausal Patients with Estrogen Receptor-Positive Metastatic Breast Cancer

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Abstract

Purpose: Abiraterone may suppress androgens that stimulate breast cancer growth. We conducted a biomarker analysis of circulating tumor cells (CTCs), formalin-fixed paraffin-embedded tissues (FFPETs), and serum samples from postmenopausal estrogen receptor (ER)⁺ breast cancer patients to identify subgroups with differential abiraterone sensitivity.

Methods: Patients (randomized 1:1:1) were treated with 1,000 mg/d abiraterone acetate + 5 mg/d prednisone (AA), AA + 25 mg/d exemestane (AAE), or exemestane. The biomarker population included treated patients ($n = 293$). The CTC population included patients with ≥ 3 baseline CTCs ($n = 104$). Biomarker (e.g., androgen receptor [AR], ER, Ki-67, CYP17) expression was evaluated. Cox regression stratified by prior therapies in the metastatic setting (0/1 vs. 2) and setting of letrozole/anastrozole (adjuvant vs. metastatic) was used to assess biomarker associations with progression-free survival (PFS).

Results: Serum testosterone and estrogen levels were lowered and progesterone increased with AA. Baseline AR or ER expression was not associated with PFS in CTCs or FFPETs for AAE versus exemestane but dual positivity of AR and ER expression was associated with improved PFS (HR 0.41 [95% confidence interval (CI), 0.16–1.07], $P = 0.070$). For AR expression in FFPETs obtained < 1 year prior to first dose ($n = 67$), a trend for improved PFS was noted for AAE versus exemestane (HR 0.56 [95% CI, 0.24–1.33], $P = 0.19$).

Conclusion: An AA pharmacodynamic effect was shown by decreased serum androgen and estrogen levels and increased progesterone. AR and ER dual expression in CTCs and newly obtained FFPETs may predict AA sensitivity.

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Translational Relevance

A subset of nonsteroidal aromatase inhibitor-resistant, estrogen receptor-positive breast cancers may be sensitive to antiandrogen drugs and thus may derive clinical benefit from the androgen biosynthesis inhibitor abiraterone acetate (AA). A biomarker analysis can be useful to identify predictive biomarkers for AA sensitivity or resistance. The effect of AA administration on serum testosterone has been well characterized but not the effect on a panel of other serum endocrine biomarkers. In our biomarker analysis, a positive pharmacodynamic effect of AA was shown by the decrease in serum estrogens and androgen and potentially by the increase in progesterone in most AA-treated patients. Despite association trends of certain biomarkers with progression-free survival (PFS), none of the biomarkers, either as single markers or in combination, could identify a subpopulation of patients with significant added clinical benefit from AA. Importantly, no association between the elevated serum progesterone induced by AA administration and PFS was observed.

Introduction

Elucidating the role of androgens in estrogen receptor (ER)⁺ metastatic breast cancer has recently been a subject of interest as androgenic signaling is predicted to be a potential mechanism of therapeutic resistance to antiestrogenic therapy (1-3). Androgens are the most prominent sex hormone in women after menopause, which may allow for androgen aromatization to estradiol and breast cancer progression through androgen receptor (AR) stimulation in a low-estrogenic environment (4). More than 60% of breast cancers express AR and androgen-regulated proteins, indicating potential androgen responsiveness (4, 5). Furthermore, exogenous AR overexpression renders tamoxifen-sensitive ER⁺ breast cancer cell lines resistant to tamoxifen, providing additional evidence for AR as a target for therapeutic augmentation of combination endocrine therapies in breast cancer (6).

As such, certain subsets of breast cancers may then be sensitive to antiandrogen strategies and thus may derive clinical benefit from the androgen biosynthesis inhibitor abiraterone acetate (AA), which has proved to be effective in hormone-driven prostate cancer (7, 8). In an early-phase, nonrandomized trial of postmenopausal ER⁺ patients with advanced breast cancer progressing on \geq two lines of endocrine therapy, AA treatment was associated with a reduction in serum androgens and estrogens (9); one patient had a confirmed partial response lasting 13.8 months, and 22% of patients had clinical benefit for \geq 24 weeks, providing preliminary evidence of efficacy of AA in advanced breast cancer (9). However, in the randomized phase II study BCA2001 of ER⁺ HER2⁻ postmenopausal breast cancer patients who had progressed on nonsteroidal aromatase inhibitors (NSAIs), no improvement in the primary endpoint of progression-free survival (PFS) was observed with the addition of AA to exemestane compared with exemestane alone (4.5 vs. 3.7 months, hazard ratio [HR] [95% confidence interval (CI)] = 0.96 [0.70–1.32]; $P = 0.79$) (10). As androgen blockage remains a potential therapeutic strategy

for a clinically defined subset of breast cancer patients, further understanding of the pathogenesis of breast cancer and mechanism of action of AA in breast cancer patients would provide the framework for individualization of this drug therapy.

We hypothesized that breast cancer tumors with active AR signaling or residual ER activity after progression on an aromatase inhibitor may derive clinical benefit from AA. We therefore conducted a biomarker analysis of study BCA2001 to analyze serum steroid concentrations for changes from baseline and to potentially identify subgroups with AA sensitivity or resistance using tumor biomarkers in archival formalin-fixed paraffin-embedded tissues (FFPETs) and circulating tumor cell (CTC) samples. We also investigated the congruency of biomarkers in these two sample types.

Patients and Methods

Study design and treatments

BCA2001 is a randomized, open-label, multicenter phase II study of 1,000 mg AA plus 5 mg prednisone daily (hereafter referred to as AA) versus AA plus prednisone with 25 mg exemestane (AAE) daily versus the control arm (25 mg exemestane alone daily [E]) in patients with postmenopausal ER⁺ HER2⁻ breast cancer after receiving NSAI (10). Patients could have had no more than two prior systemic treatments in the metastatic setting, of which one could be chemotherapy, and an Eastern Cooperative Oncology Group performance status (ECOG PS) score of ≤ 1 . Patients were stratified by the number of prior therapies in the metastatic setting (0 or 1 vs. 2) and the setting of prior letrozole or anastrozole treatment (adjuvant vs. metastatic).

The review boards at all participating institutions approved the study, which was conducted according to the Declaration of Helsinki; all patients provided written, informed consent to participate in the study.

Biomarker analysis

Several biomarkers associated with the mechanism of action of the study drugs, or with the development and progression of breast cancer, were evaluated from archival FFPETs, CTCs, and serum samples (Supplementary Table S1).

Immunohistochemistry and FISH analysis of archival FFPETs

Immunohistochemistry (IHC) and FISH analysis of archival FFPETs were conducted at Phenopath Labs (Seattle, Washington). IHC analysis was used to assess the baseline expression of ER (Thermofisher #RM-9101-S; SP1 clone), progesterone receptor (PR; Dako #M3569; PgR636 clone), HER2 (Thermo-Fisher #RM-9103-S, SP3 clone), cytochrome P450 17A1 (CYP17A1; Abcam#Ab80206), aromatase/cytochrome P450 19A1 (CYP19A1; Novus Biological #AP00001PU-N), Ki-67 (Dako, #M7240; MIB-1 clone), and AR (Dako, #M3562, Clone AR441), in FFPETs. The percentage of positively staining cells, intensity of staining (weak, moderate, strong), and presence of positive internal controls were evaluated. ER and PR positivity were assessed by scoring according to the American Society of Clinical Oncology College of American Pathologists (ASCO/CAP) guidelines (11). Expression of ER and PR at any intensity greater than 1% of tumor cells was considered positive. Expression of HER2 was assessed with a modified ASCO/CAP guideline (12). For HER2, CYP17, and CYP19, the cutpoint for positive expression was also 1% of tumor cells. To determine Ki-67 positivity, nuclear staining was scored as percentage of cells positive relative to nonreactive cells within the target. Expression of Ki-67 and AR at any intensity greater than 10% of tumor cells was considered positive.

IHC and FISH analysis of CTCs

Blood samples (40 mL) were taken on day 1 of cycles 1 and 2 and at the end of treatment (EOT) or disease progression, and shipped to the Janssen Diagnostics CRS lab for CTC enumeration (CellSearch[®]; Janssen Diagnostics, LLC, Raritan, NJ) and molecular characterization as previous described (13, 14). A three-CTC threshold is used for CTC molecular marker evaluations, whereas a five-CTC threshold separates favorable and unfavorable prognosis. Only samples with three or more evaluable CTCs were included to ensure confidence of positive versus negative biomarker determinations. Biomarkers tested in CTCs included expression of AR (Cell Signaling Technology #8428, D6F11 clone), ER (Janssen Diagnostics ER-119.3), and Ki-67 (BD Pharmingen #556027, clone B56) by immunofluorescence. For ER and Ki-67 expression analysis, the percentage of positive cells was determined by nuclear fluorescence in the phycoerythrin channel, with > 80% positive MCF-7 cells spiked into normal donor blood as a positive control. For AR expression analysis, LNCaP cells were spiked into normal donor blood as a positive control and PC-3 cells as a negative control. The cutpoints for positive expression of ER, Ki-67, and AR were the same as noted above.

A four-color FISH assay was developed for use on enriched CTC to simultaneously detect amplification of HER2 and AR genes as well as aneusomy of chromosomes X and 17. The assay uses control probes on the centromere of chromosome 17 (Kreatech SE17, Leica Biosystems) and the X chromosome (Kreatech SE-X, Leica Biosystems) to determine the overall ploidy level of the cell assessed. The Clone IDs for bacterial artificial chromosomes (BACs) utilized as probes to evaluate HER2 were RP11-62N23, RP11-94L15, and RP11-387H17. BAC clones evaluated for AR were RP11-383C12, RP11-479J1, and RP11-963N10.

Samples were considered positive for FISH, positive for HER2 and AR gene amplification if \geq three CTCs were found to contain five or more copies of the target gene.

Evaluation of serum hormone concentrations

Blood samples (15 mL) for assessment of endocrine biomarkers were taken day 1 of cycles 1 to 3 and every three cycles thereafter (e.g., day 1 cycle 6; day 1 cycle 9), and at EOT or disease progression. Analysis of androgen and estrogen concentrations was conducted according to standard operating procedures (Covance Central Laboratory Services, Indianapolis, Indiana; and Geneva, Switzerland). Samples were analyzed for testosterone, estradiol, and estrone by liquid chromatography–mass spectrometry using an ABI Sciex QTRAP® 5500 system (Applied Biosystems/MDS Analytical Technologies, Foster City, California). Progesterone serum concentrations were determined using the Beckman Coulter Access Progesterone assay (Beckman Coulter Inc., Brea, California) and the Dxi 800 instrument. Progesterone concentrations were only determined for a subset of patients, because the serum progesterone analysis was added after enrollment was initiated.

Statistical analysis

Baseline demographics and disease characteristics were compared in the biomarker or CTC populations versus the intent-to-treat (ITT) population using ANOVA and chi-squared tests. Biomarkers were summarized in each of the three arms or combinations of the three arms by calculating the mean, standard deviation, median, range, and number of samples with expression for continuous variables, and the frequency of expression of categorical biomarkers (*N* and %) for categorical variables.

For categorical variables, statistical comparisons were performed for treatment effect (AA vs. E, AAE vs. E, or AA and AAE vs. E) in biomarker positive/negative subgroups or for biomarker

effect (biomarker positive vs. negative) in treatment arms and overall population to identify patient subpopulations with better PFS. Composite biomarkers were generated by combining levels of biomarker pairs. For this composite biomarker, each individual biomarker was dichotomized to define biomarker positive and negative patient subpopulations using biomarker-specific thresholds (e.g., AR 10%, ER 1%) or medians for endocrine biomarkers. The composite biomarker was defined by evaluating individual biomarkers concurrently, resulting in four subpopulations (i.e., AR⁺ and ER⁺, AR⁺ and ER⁻, AR⁻ and ER⁺, AR⁻ and ER⁻). Association between biomarker expression and time to event was represented by reporting the *P* value and hazard ratio with 95% CI and the median time to event with 95% CI from Kaplan-Meier analysis. A Cox proportional-hazards regression analysis of PFS was performed to evaluate the treatment effect (AA vs. E, AAE vs. E) in biomarker-positive and -negative subgroups, stratified according to number of prior therapies in the metastatic setting (0 or 1 vs. 2), and setting of prior letrozole or anastrozole treatment (adjuvant vs. metastatic).

For serum endocrine markers, treatment effects were summarized by the mean, standard deviation, median, and range of expression levels, along with the change from baseline and percentage change from baseline. Longitudinal analysis was used to evaluate the effect of treatment on serum endocrine marker levels versus baseline within treatment arms. The change in serum endocrine marker levels was evaluated using a linear mixed-effects model with fixed effects for visit, treatment and the visit treatment interaction, and a random effect for patients. For CTC biomarkers, treatment effects were summarized by the count or average of total CTCs, count and percentage of biomarker-positive CTCs, change of total CTCs and biomarker-positive CTCs from baseline, and change in percentage of biomarker-positive CTCs.

CTC conversion rates were enumerated by tabulating the number of patients in each treatment arm with \geq five CTCs at baseline and $<$ five CTCs at cycle 2 or EOT instead of the \geq three CTCs

cutoff used to select patients for CTC molecular analysis. Additionally, the CTC conversion rates at cycle 2 and EOT were compared across treatment arms in biomarker (CTC or IHC AR expression) -positive versus -negative populations. Fisher's exact test was used to test the associations between treatment arms and CTC conversion rate. Expression using IHC and CTC biomarkers at baseline was compared.

Results

Patient demographics

Two hundred ninety-seven patients were enrolled in the BCA2001 trial starting in August 2011 and were randomly assigned 1:1:1 to the E ($n = 102$), AA ($n = 89$), or AAE ($n = 106$) treatment arms (Fig. 1). The primary PFS results have been presented previously (15). Of the 293 patients (E, $n = 102$; AA, $n = 87$; AAE, $n = 104$) who received treatment and comprised the biomarker population, 104 (E, $n = 37$; AA, $n = 31$; AAE, $n = 36$) were evaluable at baseline and were included in the CTC biomarker population. The remaining patients were deemed unevaluable and were excluded from the analysis due to inadequate levels of CTCs, provision of samples outside the required timeframe for CTC testing, unsatisfactory samples due to insufficient blood volume, or unreliable samples due to failure of baseline CTC testing.

The CTC biomarker populations were comparable to the ITT population with regard to patient demographics and baseline disease characteristics (10). The median age was 62 (39–81) and 63 (37–87) years in the CTC biomarker population and ITT population, respectively. Visceral metastatic disease was observed in 40% versus 46% of the patients. Altogether, 23% versus 28% of patients had two prior treatments in the metastatic setting with 66% compared to 64% of patients receiving a prior NSAID in this setting.

Biomarker analysis

A longitudinal analysis of endocrine serum markers compared within arms at several time points is presented in Fig. 2. As expected, serum testosterone was significantly decreased over time ($P < 0.0001$) in the AA and AAE treatment arms (–93.7% and –93.1% change, respectively) but not in the E arm (–15.6%; $P = 0.29$) by C2D1. AA-induced suppression of testosterone was persistent in both arms through EOT (–76.3% and –76.2%, respectively). Estradiol and estrone levels were decreased in these AA treatment arms. No statistically significant difference in the reduction of estradiol or estrone was observed between arms (data not shown).

A significant increase in serum progesterone concentrations was noted in AA-treated patients (2666.4% and 1689.0% in AA and AAE arms, respectively; $P < 0.0001$) compared with E alone (–56.6%; $P > 0.99$) by C2D1 (Fig. 2). In a separate analysis, the AA-induced increase in progesterone was not associated with PFS based on stratified Cox proportional-hazards regression analysis (data not shown). Upon further analysis of median PFS in the patients with a decrease in progesterone at C2D1 versus all patients, there was no association with shorter PFS in patients with a decrease in progesterone (data not shown).

Baseline FFPETs and CTCs were evaluated for AR and ER as single and combined biomarkers to assess the impact of AA on PFS for ER⁺ HER2[–] disease. There was a positive association with PFS for the combination of AR and ER expression in CTCs in favor of AAE versus E (HR = 0.41 [95% CI, 0.16–1.07]; $P = 0.070$) (Supplementary Table S2). Evaluated as single biomarkers, a positive expression of AR (Fig. 3A and 3B) or ER in baseline CTCs or in the FFPETs was not associated with an improvement in PFS with AA or AAE versus E. No association between negative AR expression and improved PFS in the three arms was also noted (Supplementary Fig. S1A and S1B). Loss of ER or AR expression in CTCs was also not associated with a difference in PFS with AA or AAE versus E. For patients with FFPETs obtained < 1 year prior to first dose ($n = 67$) (Fig. 3C), positive AR expression was associated

with an improvement in PFS for AAE versus E (HR = 0.56 [95% CI, 0.24–1.33]; $P = 0.19$).

Negative AR expression in these same recent FFPETs was not associated with an improvement in PFS for AAE versus E (Supplementary Fig. S1C). For completeness, we evaluated the sensitivity of the results to the threshold used to dichotomize low versus high expression. We found that the results were invariant to the specific threshold chosen (data not shown).

Additional biomarkers were evaluated for a potential association with PFS (Supplementary Table S2). A longer median PFS was observed for positive Ki-67 expression in CTC samples (defined as $\geq 10\%$ staining) in AAE-treated patients (3.7 months, HR = 0.26 [95% CI, 0.11–0.64]; $P = 0.003$), and when AA and AAE arms were combined (3.5 months, HR = 0.36 [95% CI, 0.17–0.76]; $P = 0.007$) compared with the E arm (1.9 months). Conversely, a statistically significant improvement in PFS with AAE was not noted for positive Ki-67 expression ($\geq 10\%$) in FFPETs obtained at diagnosis. Patients with low serum estrone levels at baseline in the AAE and combined AA and AAE groups also showed an improvement in PFS over patients in the E arm (4.2 months, HR = 0.67 [95% CI, 0.43–1.04]; $P = 0.07$ vs. 3.7 months, HR = 0.71 [95% CI, 0.48–1.05]; $P = 0.09$ vs. 1.9 months, respectively). No improvement in PFS was associated with expression of CYP17 or CYP19 in FFPETs.

With regard to the post-baseline CTC analysis (Table 1), all patients' CTCs were negative for ER amplification by FISH at baseline and post-baseline. There were significantly lower CTC counts in the AA arms compared with E alone at cycle 2 day 1 ($P = 0.0075$) but not at EOT ($P = 0.95$). No difference in CTC AR expression or CTC ER expression post-baseline was observed between treatment groups.

Discordance in CTC and FFPET expression of biomarkers was observed. In total, 11 of 58 (19%), 25 of 77 (33%), and 7 of 44 patients (16%) whose breast cancers were positive for

expression of AR, ER, and Ki-67, respectively, in FFPETs were negative on CTC analysis (Table 2). An observed discordance occurred in similar frequencies for all three of these biomarkers.

Loss of ER expression may occur over time such that FFPETs initially characterized as ER⁺ may be ER⁻ at the time of treatment for metastatic disease. Our analysis demonstrated that 33% and 16% of initially positive breast cancer had lost ER or AR, respectively, in their CTCs. We evaluated whether loss of ER or AR is associated with worse outcomes by evaluating PFS differences between biomarker-positive and -negative patients for each of the AA treatment arms versus E. No significant differences in outcomes were observed (data not shown).

Discussion

This is the first report of a biomarker assessment of AA-treated postmenopausal ER⁺ advanced breast cancer patients based on serum endocrine analysis, FFPETs, and CTCs. The goal of this study was to assess baseline biomarkers that were highly associated with treatment response for stratification strategies and to track molecular and endocrine changes throughout the course of treatment. If a positive marker could be identified, uncertainty in predicting clinical benefit from current therapeutic options, which often leads to ineffective treatment of disease, can be avoided. Biomarker-driven therapeutic decision-making can potentially offer valuable information on surrogate markers for disease progression and treatment in the clinical setting.

A positive pharmacodynamic effect of AA was shown by the decrease in serum androgen levels as well as estrogen levels (Fig. 2). Since CYP17 is upstream from aromatase in the steroid synthesis pathway, theoretically, AA should inhibit production of estrogens. Data from this study showed that AA decreased circulating estradiol and estrone to a level comparable to E alone.

A trend of sensitivity was observed in certain subsets of biomarkers analyzed in this study but the improvement in terms of longer PFS was generally not clinically significant. Baseline AR and ER expression in CTCs or FFPETs was not observed to be associated with improved PFS with AA or AAE versus E, though baseline AR and ER dual expression in CTCs may have an association with improved PFS (Supplementary Table S2).

We hypothesized that loss of ER expression in CTCs might predict resistance to AA because of insensitivity of ER⁻ tumors to hormonal therapy. However, even though loss of ER was observed in a subset of CTCs, it was not predictive of worse PFS with AA. Potential reasons for this outcome include discordance between diagnostic and CTC samples, or crosstalk between AR and ER signaling.

Some limitations to analysis of CTC may have affected interpretations made from this study. One limitation is the small number of patients who have sufficient numbers of CTCs for analysis. In this study, only 39% (104/266) of the treated patients who provided CTCs had three or more CTCs at baseline for inclusion in the population for biomarker analysis. We found that there was 81% concordance between AR positivity in FFPE versus CTC, whereas 32.5% of CTC samples lost ER expression. Loss of ER expression in CTCs can be attributed to a number of potential factors, including repopulation of tumor cells in response to various treatments after FFPETs were taken, intra- and inter-lesion heterogeneity of ER expression, lack of sufficient evaluable CTCs, and differences in IHC and CTC staining methods. Well-controlled future studies are needed to determine the exact cause of the discordance of ER status.

Positive AR expression in FFPETs obtained < 1 year prior to the first dose was associated with improved PFS in the AAE arm versus E (Fig. 3C), while AR expression assessed in all the

archival FFPETs (some as old as 25.67 [0–25.67] years) was not. Samples collected more recently could have had a greater correlation of PFS with AA treatment due to antigen preservation, more recent samples being more representative of current tumor status, and/or differences in sample quality (signal may not be stable in older samples). In addition, a higher proportion of metastasis in recently collected samples compared with older samples (OR = 3.26, $P = 0.005$) could have affected these findings.

The methods used in these analyses also did not allow for multiplex testing, which would have enabled multiple biomarkers to be measured simultaneously (16). This may affect data accuracy and appearance of discordance, as only a few individual molecular markers provide adequate sensitivity for CTC detection. Furthermore, multiple individual assays require sufficient CTC sample for each analysis, which limits the biomarker population to those patients who have higher levels of CTCs. Because of this, it is possible that patients were misclassified as biomarker-negative by CTC testing based on defined cutpoints because there were not enough CTCs detected and available for determination of status of individual CTCs, contributing to discordance.

The lack of an observed positive association between AR expression and improved PFS with AA may be due to AA-induced progesterone production (Fig. 2). Progesterone potentially acts as an agonist that induces growth and survival of ER⁺ breast cancer. Importantly, in this evaluation of the association of baseline progesterone with PFS as continuous variable, patients with higher baseline progesterone concentrations had shorter PFS in all arms. The prognostic value of baseline progesterone, however, diminished after initiation of treatment in all arms. Evaluation of the association of elevated progesterone levels with PFS in samples on-treatment and post-treatment with AA was not feasible, because almost all patients treated with AA had

elevated serum progesterone levels. Progesterone levels were observed to be nominally lowered by 56.6% in the E arm at C2D1. This decrease of progesterone, however, was not statistically significant and was likely due to an outlier and not a real treatment effect.

In conclusion, our analyses show that AA treatment decreased serum testosterone and estrogen levels but increased progesterone concentrations in ER⁺ HER2⁻ NSAI-resistant metastatic breast cancer patients. Baseline AR and/or ER expression in CTCs or archival FFPETs was not associated with improved PFS for AA treatment arms versus E. However, an analysis of recent FFPETs, which may be more representative of the current metastatic disease biology, suggested that AR expression predicted for improved PFS with AA treatment, potentially due to the enrichment of AR in metastatic breast cancers that had been previously treated with a NSAI (6). Due to the small sample size, the hypotheses generated in these exploratory analyses need to be confirmed in further studies. However, due to a lack of significant improvement in clinical outcomes in the primary phase II trial (15), a phase III trial of the combination of AA and E in this breast cancer patient population was not pursued.

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Table 1. Post-baseline analysis of treatment effect on CTC markers

Biomarker	Visit	Treatment	N	Mean	Differential estimate (95% CI)	P value
CTC counts	C1D1	E	36	45.89	–	–
		AA	31	25.10	–	–
		AAE	34	68.24	–	–
		AA and AAE	65	47.66	–	–
	C2D1	E	25	81.89	–	–
		AA	28	27.25	–1.10 (–1.92 to –0.29)	0.0075
		AAE	31	24.35	–1.21 (–2.02 to –0.43)	0.0026
		AA and AAE	59	25.73	–1.16 (–1.89 to –0.48)	0.0012
	EOT	E	20	48.68	–	–
		AA	17	47.18	–0.03 (–0.92 to 0.88)	0.95
		AAE	20	77.66	0.47 (–0.39 to 1.33)	0.28
		AA and AAE	37	63.65	0.27 (–0.51 to 1.00)	0.48
CTC AR	C1D1	E	25	43.96	–	–
		AA	25	45.20	–	–
		AAE	30	35.70	–	–
		AA and AAE	55	40.02	–	–
	C2D1	E	19	51.74	–	–
		AA	18	45.50	–6.24 (–24.14 to 11.67)	0.49
		AAE	25	41.48	–10.26 (–26.83 to 6.31)	0.22
		AA and AAE	43	43.16	–8.57 (–23.57 to 6.42)	0.26
	EOT	E	14	41.07	–	–
		AA	15	49.87	8.80 (–12.17 to 29.76)	0.41
		AAE	18	28.39	–12.68 (–32.78 to 7.42)	0.21
		AA and AAE	33	38.15	–2.92 (–20.91 to 15.07)	0.75
CTC ER	C1D1	E	25	27.84	–	–
		AA	25	27.64	–	–
		AAE	27	25.04	–	–
		AA and AAE	52	26.29	–	–

C2D1	E	12	19.75	–	–
	AA	12	29.08	9.33 (–9.31 to 27.98)	0.32
	AAE	11	26.09	6.34 (–12.72 to 25.40)	0.51
	AA and AAE	23	27.65	7.90 (–8.36 to 24.16)	0.33
EOT	E	13	28.08	–	–
	AA	13	37.54	9.46 (–13.70 to 32.62)	0.42
	AAE	18	28.17	0.09 (–21.40 to 21.58)	0.99
	AA and AAE	31	32.10	4.02 (–15.49 to 23.53)	0.68

AR, androgen receptor; C1D1, cycle 1 day 1; C2D1, cycle 2 day 1; CI, confidence interval; CTC, circulating tumor cell; ER, estrogen receptor; EOT, end of treatment..

Table 2. Discordance of CTC at baseline and FFPET at diagnosis

		-CTC	+CTC
AR, <i>n</i> (%)	– by IHC	4 (23.5)	13 (76.5)
	+ by IHC	11 (19.0)	47 (81.0)
ER, <i>n</i> (%)	– by IHC	–	–
	+ by IHC	25 (32.5)	52 (67.5)
Ki-67, <i>n</i> (%)	– by IHC	7 (36.8)	12 (63.2)
	+ by IHC	7 (15.9)	37 (84.1)

AR, androgen receptor; CTC, circulating tumor cell; ER, estrogen receptor; FFPET, formalin-fixed paraffin-embedded tissue; IHC, immunohistochemistry.

Cutpoints for positive expression: AR, 10%; ER, 1%; Ki-67, 10%.

Figure Legends

Figure 1. CONSORT diagram. FFPETs were collected from all patients. E, exemestane; AA, abiraterone acetate; AAE, abiraterone acetate and exemestane; CTC, circulating tumor cell; ITT, intent-to-treat population.

Figure 2. Longitudinal analysis of serum endocrine markers. (A) Testosterone. (B) Progesterone. (C) Estradiol. (D) Estrone. E, Exemestane; AA, abiraterone acetate; AAE, abiraterone acetate and exemestane; C1D1, cycle 1 day 1; C2D1, cycle 2 day 1; C3D1, cycle 3 day 1; C6D1, cycle 6 day 1; C9D1, cycle 9 day 1; EOT, end of treatment.

Figure 3. Comparison of associations of the positive baseline expression of androgen receptor (AR) ($\geq 10\%$) with progression-free survival (PFS). (A) CTCs. (B) FFPETs by immunohistochemistry. (C) FFPETs collected < 1 year before first dose. Positive ($\geq 10\%$). E, exemestane; AA, abiraterone acetate; AAE, abiraterone acetate and exemestane.

Figure 1

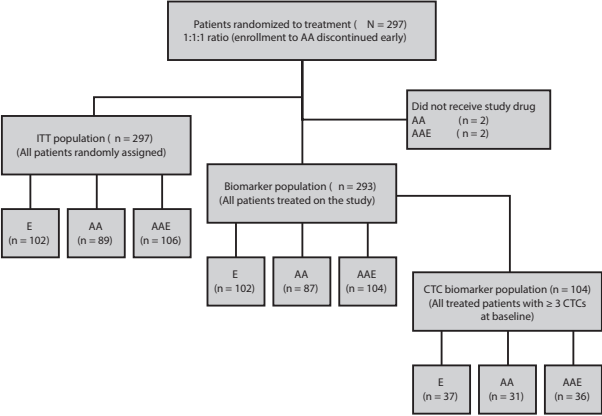


Figure 2

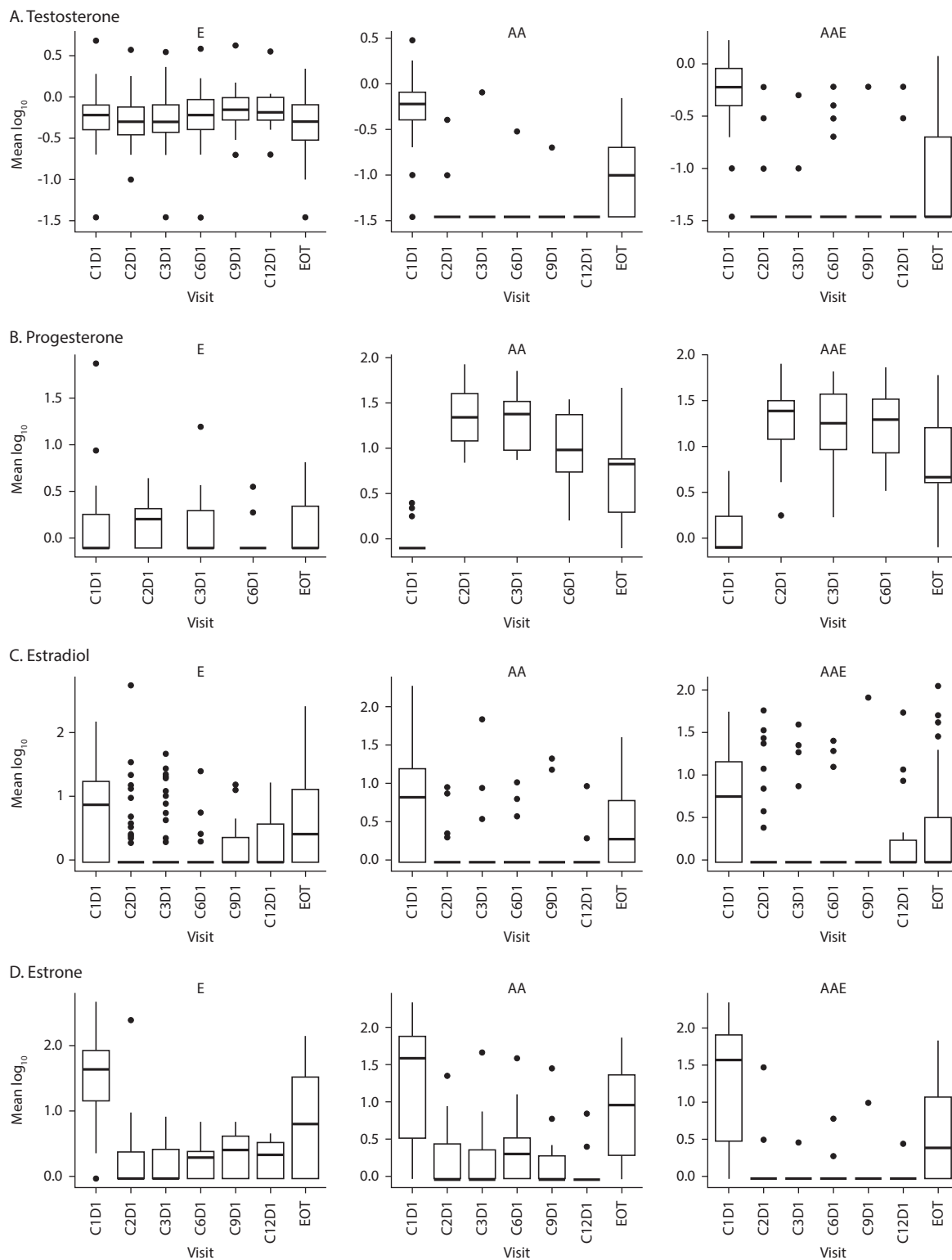


Figure 3

